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(54) Antimicrobial compositions and pharmaceutical preparations thereof.

(57) Antimicrobial compositions containing effective ingredients composed of antimicrobial peptides derived from horseshoe crab, their derivatives or pharmaceutically acceptable salts and a β-lactam antibiotic or chloramphenicol antibiotic.

Defensive agents against opportunistic infections containing the composition as effective ingredients.

The compositions exhibit bactericidal effect against methicillin resistant Staphylococcus aureus spp. (MRSAs) at low concentrations and are useful as antimicrobial agents, particularly for the prevention and treatment of opportunistic infections.

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difficult.

The peptides of the present invention includes not only natural antimicrobial peptides derived from horseshoe crab but also their derivatives having one to several modified amino acids such as substitution, deletion or elongation exhibiting similar antimicrobial activity (hereinafter abbreviated as derivatives). These derivatives include peptides which have been replaced their basic and/or aromatic amino acids with the other basic and/or aromatic amino acids, respectively. (see European Patent Application (A1) No. 0502188).

Above mentioned antimicrobial peptides can be extracted from the hemocytes of horseshoe crab such as Limulus polyphemus available in U.S.A., Tachypleus tridentatus available in China and Japan, Tachypleus gigas available in Thailand and Malaya Peninsula and Carcinoscorpius rotundicauda available in Thailand and Malaya Peninsula by known methods. These peptides can be obtained by known peptide synthetic methods such as solid phase synthesis and liquid phase synthesis, or by genetic engineering methods using transformed or transfected microorganisms and animal cells having gene DNA coding for said peptides. Furthermore, said peptides may be acid amide form at the C-terminal amino acid.

The antimicrobial peptides isolated from horseshoe crab contain many basic amino acids such as arginine and lysine showing basic property and may form salts with acids.

The present invention can utilize such pharmaceutically acceptable salts as hydrochloride, sulfate, nitrate, phosphate, formate, acetate, lactate, oxalate, maleate, fumarate, succinate, trifluoroacetate, p-toluenesulfonate, methanesulfonate, and etc.

The β-lactam antibiotics used in the present invention include cephalosporins and penicillins and any known antibiotics can be used for the present invention. Cephalosporin antibiotics such as cefazolin, cephalexin, cefamandole, cefoxitin, cefmetazole, cefotaxime and cefotetan, and penicillin antibiotics such as ampicillin, hetacillin, talampicillin, bacampicillin and carbenicillin can be illustrated.

Furthermore, the present invention may utilize chloramphenicol.

The antimicrobial compositions of the present invention composed of antimicrobial peptides isolated from horseshoe crab, their derivatives or pharmaceutically acceptable salts and a β-lactam antibiotic or chloramphenicol antibiotic exhibit potent antimicrobial activities against Gram positive bacteria including MRSAs and Gram negative bacteria at low concentrations. Therefore, the compositions are useful as antimicrobial medical agents for the prevention and treatment of infections of respiratory tract, wounds and urogenital tract, and otorhinolaryngological and ophthalmological infections, and sepsis.

The compositions may be used for the prevention and treatment of stomatitis, periodontitis, dental caries and so forth caused by oral microorganisms.

The compositions are particularly effective against MRSAs at low concentrations, thus can be applied for the prevention and treatment of patients in critical condition caused by MRSA infections of deeper lying organs and opportunistic infectious diseases of immunocompromised patients due to the dosage of anticancer agents or immunosuppressive agents.

Furthermore, above mentioned compositions may be used for gargles and disinfectants for the prevention of nosocomial infections of MRSAs from infected patients or carriers to the other hospitalized patients and members of the institute free from MRSA.

The antimicrobial compositions of the present invention can be used to prepare various pharmaceutical preparations using conventional carriers, fillers, binders, disintegrators, lubricants, sweeteners and so forth by known methods. The resultant compositions may be administered orally as solid preparations such as tablets, capsules, granules, powder preparations and troches, and liquid preparations such as syrup and elixirs. The compositions can be administered parenterally as injections, for example intravenous and intramuscular injections, or spray forms such as aerosol preparations. Furthermore, the compositions may take forms of topical preparations such as suppositories, ointments and cataplasms.

In the compositions of the present invention, the weight ratios of the antimicrobial peptides derived from horseshoe crab, their derivatives or pharmaceutically acceptable salts and β-lactam antibiotics or chloramphenicol antibiotics are generally 1:0.5 to 1:50, but may be modified according to the properties of the antibiotics. The resultant compositions are administered preferably at doses of 0.1-100 mg/kg/day in several portions though the doses may vary with the symptoms and ages of patients. The compositions exhibit minimum inhibitory antimicrobial activities at doses of 1/2 or lower to those of single administration of the antimicrobial peptides derived from horseshoe crab with less toxic adverse effects.

The antimicrobial effect of the composition of the present invention will be shown by the following experiments.

results against MRSA No. 3-50 strain are shown in the following Tables.

(i) Combinations of tachyplesin I (TAC-I) and cefazolin (CEZ)

Table 1

CEZ (μg/ml)	TAC-I (μg/ml)			
	0	0.4	0.8	1.6
0	+	+	+	+
10	+	+	+	+
20	+	+	+	-
40	-	-	-	-

In the Table, + shows growth
- shows no growth

(ii) Combinations of tachyplesin I (TAC-I) and ampicillin (ABPC)

Table 2

ABPC (μg/ml)	TAC-I (μg/ml)			
	0	0.4	0.8	1.6
0	+	+	+	+
10	+	+	+	+

- shows no growth

(iii) Combinations of tachyplesin I (TAC-I) and chloramphenicol (CP)

Table 3

CP (μg/ml)	TAC-I (μg/ml)			
	0	0.4	0.8	1.6
0	+	+	+	+
5	+	+	+	+
10	+	+	+	-
20	+	+	+	-

In the Table, + shows growth
- shows no growth

As shown in the above Tables, concurrent administration of tachyplesin I and cefazolin, ampicillin or chloramphenicol exhibited remarkably enhanced antimicrobial effect at a concentration of 1.6 μg/ml of tachyplesin I in comparison with that of 3.2 μg/ml for single administration of tachyplesin I. Combinations of polyphemusin II and above mentioned antibiotics were also investigated and the combinations and single administrations showed MIC of 1.6 and 3.3 μg/ml, respectively. Thus marked synergistic effects in the

2. The composition according to claim 1, wherein the antimicrobial peptide isolated from horseshoe crab hemocyte is at least one peptide selected from a group of tachyplesin I, tachyplesin II, tachyplesin III, polyphemusin I, polyphemusin II and gigasin II.

3. The composition according to claim 1, wherein the β-lactam antibiotic is at least one cephalosporin antibiotic selected from a group of cefazolin, cephalexin, cefamandole, cefoxitin, cefmetazole, cefotaxime and cefotetan.

4. The composition according to claim 1, wherein the β-lactam antibiotic is at least one penicillin antibiotic selected from a group of ampicillin, hetacillin, talampicillin, bacampicillin and carbenicillin.

5. A pharmaceutical composition comprising an antimicrobial peptide isolated from horseshoe crab hemocyte, its derivative or its pharmaceutically acceptable salt, and in mixture with at least one antibiotic selected from a group of β-lactam antibiotic and chloramphenicol antibiotic.

6. The antiopportunistic infection composition comprising an antimicrobial peptide isolated from horseshoe crab hemocyte, its derivative or its pharmaceutical acceptable salt, and in mixture with at least one antibiotic selected from a group of β-lactam antibiotic and chloramphenicol antibiotic.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 93105145.2

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP - A - 0 384 410 (BANYU PHARMACEUTICAL CO., LTD) * Abstract; claims 1,4,6; page 2, lines 10-32,42-44; page 3. lines 15-29 *	1,3,4, 5,6	A 61 K 37/02 A 61 K 31/165 A 61 K 31/43 A 61 K 31/545
A	CHEMICAL ABSTRACTS, vol. 77, no. 3, July 17, 1972 Columbus, Ohio, USA I. MARTIN et al. "Synergism between phosphonomycin and other antibiotics", page 109, column 1, abstract-no. 14 802q & An. Inst. Farmacol. Espan. 1970 (Pub. 1971) 19, 341-50 (Span.)	1,4,5, 6	
A	CHEMICAL ABSTRACTS, vol. 115, no. 8, August 26, 1991 Columbus, Ohio, USA H. NAKAJIMA et al. "Gigasin II (peptide) from Tachypleus gigas and its use as microbicide", page 475, column 2, abstract-no. 78 892e & Jpn. Kokai Tokkyo Koho JP 02,270,897 (90.270,897)	1,2,5, 6	TECHNICAL FIELDS SEARCHED (Int. Cl.5) A 61 K 37/00 A 61 K 31/00
A	PATENT ABSTRACTS OF JAPAN, unexamined applications, C field, vol. 14, no. 403, August 31, 1990 THE PATENT OFFICE JAPANESE GOVERNMENT page 160 C 753 * No. 2-152 987 (TAIYO FISHERY CO LTD) *	1,2,5, 6	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	21-05-1993	MAZZUCCO

EPO FORM 1503 03.82 (P0401)

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

Information on patent family members

International Application No
PCT/CA 01/00918

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		US 5610139 A	11-03-1997

International application No.
PCT/CA 01/00918

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 36
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

International Application No. PCT/CA 01 00918

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 36

Present claim 36 relates to a compound defined by reference to a desirable characteristic or property, namely made of two antiparallel beta strands and comprising a beta hairpin loop and having antimicrobial activity.
whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for the subject matter of claim 36.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.